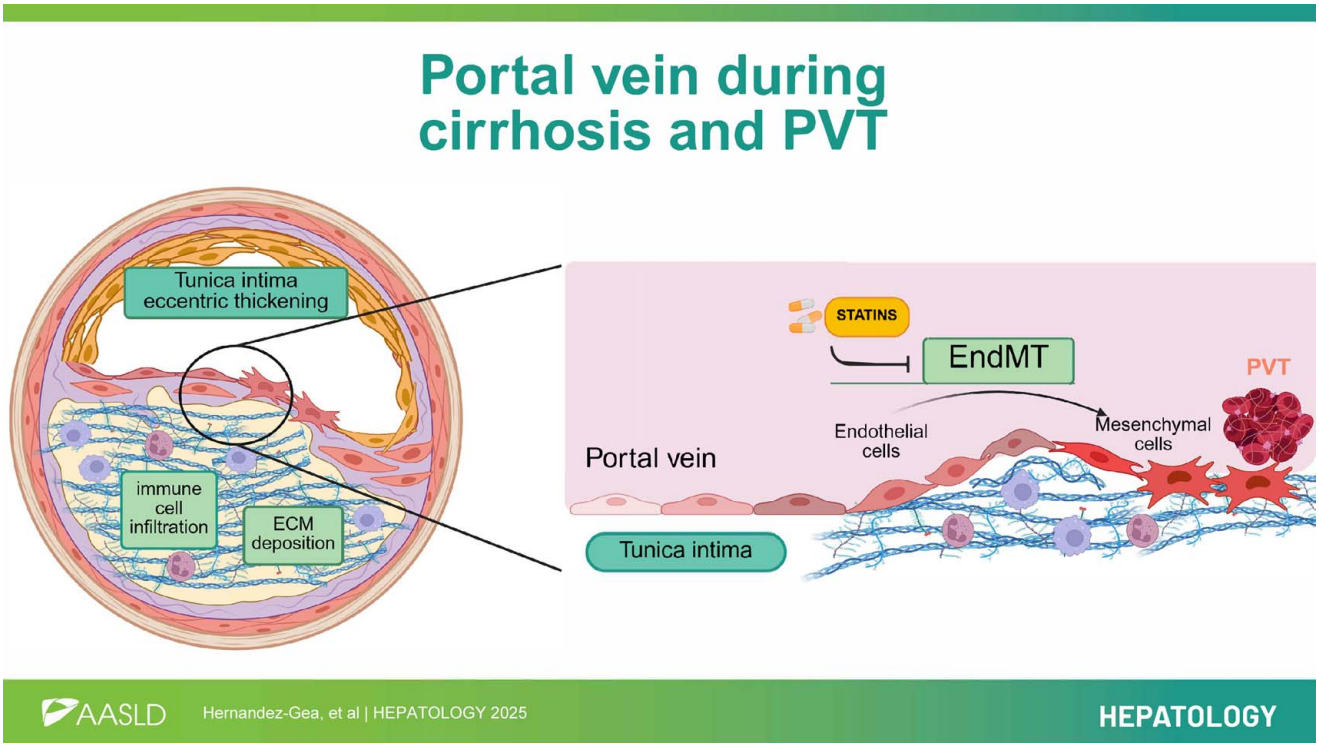
Endothelial-to-mesenchymal transition: A targetable mechanism that contributes to portal vein thrombosis in cirrhosis

VISUAL ABSTRACT



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ORIGINAL ARTICLE

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Endothelial-to-mesenchymal transition: A targetable mechanism that contributes to portal vein thrombosis in cirrhosis

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Abbreviations: CH_PVECs, portal vein endothelial cells from cirrhotic patients without PVT; CT, control; CT_PVECs, portal vein endothelial cells from controls; DEG, differentially expressed genes; DGIdb, Drug–Gene Interaction Database; EC, endothelial cell; ECM, extracellular matrix; EndMT, endothelial-to-mesenchymal transition; FFPE, formalin-fixed paraffin-embedded; GSEA, Gene Set Enrichment Analysis; GVD, graft vascular disease; H&E, hematoxylin and eosin; LT, liver transplantation; PHT, portal hypertension; PLD, polycystic liver disease; PPVL, partial portal vein ligation; PV, portal vein; PVEC, portal vein endothelial cell; PVT_PVECs, portal vein endothelial cells from cirrhotic patients with PVT.

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Abstract

Background and Aims: Portal vein thrombosis (PVT) is a frequent complication of cirrhosis, yet its pathophysiology remains elusive. Unlike thrombosis in other vascular beds, anticoagulation often fails to completely recanalize PVT, highlighting the need for greater mechanistic insight and alternative therapeutic approaches. Our study aimed to characterize the structural and cellular changes in the portal venous vascular bed to identify novel physiologically driven therapeutic targets.

Approach and Results: Portal vein tissue specimens from explanted livers of patients with cirrhosis with and without PVT and from liver donors were histologically analyzed. PVT is associated with a remarkable eccentric thickening of the tunica intima due to accumulation of extracellular material and tunica intima increased cellularity. Transcriptomic analysis of human portal vein endothelial cells (PVEC) from patients with PVT identified the endothelial-to-mesenchymal transition (EndMT) pathway upregulated in PVECs from patients with PVT. In silico drug repurposing analysis identified statins as potential agents targeting these alterations. In vitro treatment with statins attenuated hallmarks of EndMT in primary human PVECs.

Conclusions: In cirrhosis, the portal vein endothelium undergoes maladaptive remodeling marked by eccentric intimal hyperplasia, driven by ECM accumulation and infiltration of inflammatory and myofibroblast-like cells. This process narrows the lumen and may promote PVT. EndMT plays a key role in this transformation and emerges as a novel and promising therapeutic target. Notably, statins can attenuate EndMT, highlighting its potential in PVT treatment.

Keywords: cirrhosis, endothelial cells, endothelial dysfunction, portal hypertension, portal vein, statins, thrombosis, transcriptomic

INTRODUCTION

The extrahepatic portal vein (PV) is a critical site of vascular injury in cirrhosis and undergoes significant changes, including altered flow, pressure, collateral formation, and thrombosis, yet it remains understudied. While intrahepatic vascular remodeling and liver sinusoidal endothelial cells (LSECs) dysfunction are well characterized, molecular alterations within the extrahepatic vasculature are largely unknown. The mechanisms driving these changes, and their impact on disease progression, remain unclear, highlighting a critical gap in our understanding of cirrhosis-related PV dysfunction.

Patients with cirrhosis frequently develop portal vein thrombosis (PVT),[1] a rare event in the absence of liver disease but a major clinical challenge in advanced cirrhosis, where its prevalence reaches up to 35%.[2] PVT is particularly concerning in liver transplantation (LT),[3–6] the only definitive treatment for end-stage liver disease, as it increases graft complications, postoperative morbidity, and mortality.[7,8]

Despite its clinical significance, the pathophysiology of PVT remains poorly understood, and treatment is largely limited to anticoagulation. However, anticoagulation is often ineffective,[9] with up to 60% of cases failing to achieve complete recanalization[10,11] and thrombus progression occurring in 14% despite treatment.[12] In addition, anticoagulation poses significant risks, including gastrointestinal and cerebral hemorrhage. The high failure rate and associated risks underscore the urgent need for novel therapies and a deeper understanding of PVT mechanisms.

This distinct response to anticoagulation may stem from the unique pathophysiology of the PVT, as the composition of the thrombus in the PV likely differs from that of thrombosis in other venous territories.[13] In addition, the role of the Virchow triad, responsible for venous thrombosis, remains only partially understood in

PVT during cirrhosis, further complicating its mechanistic framework. Studies have failed to demonstrate a local hypercoagulable environment in the splanchnic territory,[14–17] and the slow portal flow—associated with increased PVT risk—is insufficient alone to trigger thrombosis.[18–20] In that scenario, while endothelial dysfunction has been proposed as a key factor in PVT development,[14,21,22] its anatomical inaccessibility poses a significant challenge for sample collection and real-time monitoring of endothelial changes. Accessing the PV requires its direct puncture, an invasive procedure associated with substantial risks. Given these ethical and safety concerns, routine sampling for research purposes is not feasible outside the context of LT. As a result, research on the liver endothelium has been limited to studies of isolated LSECs, while the isolation of portal vein endothelial cells (PVECs) has only recently been achieved.[23] In addition, the lack of suitable animal models for translational research hinders progress, as current portal hypertension (PHT) models do not fully replicate the human portal circulation microenvironment or the rebalanced coagulation system in humans, and they do not spontaneously develop PVT.

Our study aims to bridge this gap by examining the PV endothelium at both macroscopic and microscopic levels to better understand PV endothelium changes under cirrhosis and PVT complications. Given that PVECs have never been studied before, our research offers a novel perspective by focusing on the molecular mechanisms behind PV endothelial dysfunction in patients with cirrhosis to identify new signaling pathways that could uncover therapeutic targets, paving the way for innovative PVT treatments.

METHODS

Study population

To perform all experiments in the current study, we used 3 independent cohorts of patients' samples, all obtained in the context of liver transplant—the sole clinical context in which obtaining such samples is feasible. We prospectively collected extrahepatic portal vein for histological analysis (cohort A) and isolation of PVECs (cohort B). We also evaluated stored portal vein wall specimens from the liver hilum after liver transplant (cohort C, retrospective, described in Supplemental Material, http://links.lww.com/HEP/K282). In all cases, samples of the portal vein were obtained from thrombus-free regions. Even in patients with PVT, and despite intraoperative confirmation of thrombus presence, tissue specimens were never collected directly from thrombus-containing sites.

The baseline clinical characteristics of the 3 cohorts are detailed independently in Table 1 and Supplemental Tables S1–S4, http://links.lww.com/HEP/K282. A direct comparison of these characteristics across the 3 cohorts is provided in Supplemental Table S5, http://links.lww.com/HEP/K282.

Patient cohort A

Histological analysis was performed on extrahepatic portal vein wall samples obtained from consecutive LT recipients and donors between 2019 and 2023. As a control group (CT), remnant portal vein samples were collected from liver grafts donated for LT during the same period. Tissues were rapidly fixed in either formalin or PAXgene fixatives and processed to obtain paraffin-embedded (FFPE) blocks.

The study group included patients undergoing LT for cirrhosis, regardless of etiology, while excluding those with microvascular or macrovascular hepatocellular carcinoma (HCC) invasion. Portal vein samples from this group were further sub-classified based on the patients' history of PVT. Samples from patients with PVT were labeled as the PVT-group, while those from patients with cirrhosis without PVT are referred to as CH-patients. For all PVT-patients, we recorded whether PVT was diagnosed before LT or incidentally at the time of LT. All PVT diagnosed before LT were characterized by contrast-enhanced computed tomography or magnetic resonance imaging and classified as partial or complete following the Baveno VII Consensus[9] and VALDIG criteria.[24] The degree of main portal trunk occlusion was also examined in the surgical report of portal vein dissection during LT for all patients. Clinical variables were collected from clinical records. All patients provided written informed consent for the use of biological samples obtained at LT.

The study was approved by the ethical committee of Hospital Clínic de Barcelona (HCB/2018/1246) and the Biobank (A2-C21020) in accordance with the International Guideline for Ethical Review of Epidemiological Studies and principles of the Declaration of Helsinki.

Methodology for histological characterization and immunohistochemical staining protocols are described in Supplemental Material, http://links.lww.com/HEP/K282. Histological analysis and quantifications were conducted by 2 expert pathologists (María Luisa Botero and Carla Montironi).

Patient cohort B

Portal vein (PV) wall samples were prospectively collected for PVEC isolation from patients with cirrhosis, with or without PVT, undergoing LT between 2021 and 2023 at the Hospital Clinic de Barcelona (cohort B).

We excluded patients with active viral (HCV, HBV, HDV, or HIV) infection at the time of surgery, as well as those with micro or macrovascular invasion of HCC in the PV.

TABLE 1 Cohort B: Patients' characteristics

	CH group n = 12	PVT group n = 5	*p*
Age, years—median (IQR)	60 (53–64)	61 (59–63)	0.916
Gender female—number of patients (%)	3 (25%)	0 (0%)	0.515
BMI—median (IQR)	26 (23–32)	28 (26–33)	0.574
Obesity—number of patients (%)	5 (42%)	3 (60%)	0.620
Diabetes mellitus—number of patients (%)	4 (33%)	2 (40%)	0.999
Arterial hypertension—number of patients (%)	5 (42%)	0 (0%)	0.245
Inherited/acquired thrombophilia—number of patients (%)	0 (0%)	1 (14%)	0.333
Etiology of liver disease—number of patients (%)			
HCV	2 (17%)	0 (0%)	
HBV/HBV–HDV	0 (0%)	0 (0%)	
Alcohol	9 (75%)	3 (60%)	0.169
Cholestatic + autoimmune	1 (8%)	0 (0%)	
MASH	0 (0%)	2 (40%)	
Cryptogenic	0 (0%)	0 (0%)	
Child–Pugh score—number (%)			
A	3 (25%)	0 (0%)	0.279
B	3 (25%)	3 (60%)	
C	6 (50%)	2 (40%)	
MELD—median (IQR)	19 (13–25)	19 (19–25)	0.596
MELD-Na—median (IQR)	21 (13–27)	25 (19–27)	0.634
HCC (previous/still active at LT)—number of patients (%)	4 (33%)	Previous 1 (20%)	0.582
	2 (17%)	Active 0 (0%)	0.638
Varices—number of patients (%)	10 (71%)	4 (57%)	0.638
Ascites—number of patients (%)	7 (58%)	4 (80%)	0.600
Encephalopathy—number of patients (%)	9 (75%)	5 (100%)	0.515
Splenomegaly—number of patients (%)	11 (92%)	4 (80%)	0.496
Platelets, $\times 10^9$/L—median (IQR)	77 (56–88)	53 (53–66)	0.459
NSBB—number of patients (%)	6 (50%)	5 (100%)	0.799
Statins—number of patients (%)	1 (8%)	0 (0%)	0.361
Rifaximin—number of patients (%)	7 (58%)	2 (40%)	0.490
Anticoagulant—number of patients (%)	0 (0%)	2 (40%)	0.074
Anti-platelet drugs—number of patients (%)	1 (8%)	0 (0%)	0.088

Abbreviations: BMI, body mass index; CH, chronic hepatitis; LT, liver transplantation; MASH, metabolic dysfunction–associated steatohepatitis; MELD-Na, Model for End-Stage Liver Disease—Sodium; NSBB, nonselective beta-blocker.

As controls, initial efforts to use portal vein samples from liver graft donors were unsuccessful, as these exhibited poor endothelial cell viability due to ischemia-reperfusion injury following cold preservation during transplantation (data not shown.[23]) To address this limitation, we selected PV samples from patients with polycystic liver disease (PLD) who were candidates for transplantation solely due to symptomatic massive hepatomegaly, with preserved hepatic function and in the absence of PHT, as the most appropriate control group. Importantly, these patients typically do not present with vascular abnormalities, meaning that, in cases where PHT is absent, the portal vein remains structurally and functionally preserved, resembling its physiological condition. Notably, no other clinical contexts allow for ethical and feasible acquisition of portal vein tissue for research purposes.

Patient cohort C details can be found in the Supplemental Material, http://links.lww.com/HEP/K282.

Statistical analysis

Statistical analysis was performed using R software version 4.3.1 for Windows. Continuous variables, provided as raw data and expressed in boxplots as median and interquartile range (IQR), were compared using the Student *t* test when comparing 2 groups where adequate. Categorical variables, expressed as absolute numbers and percentages, were compared

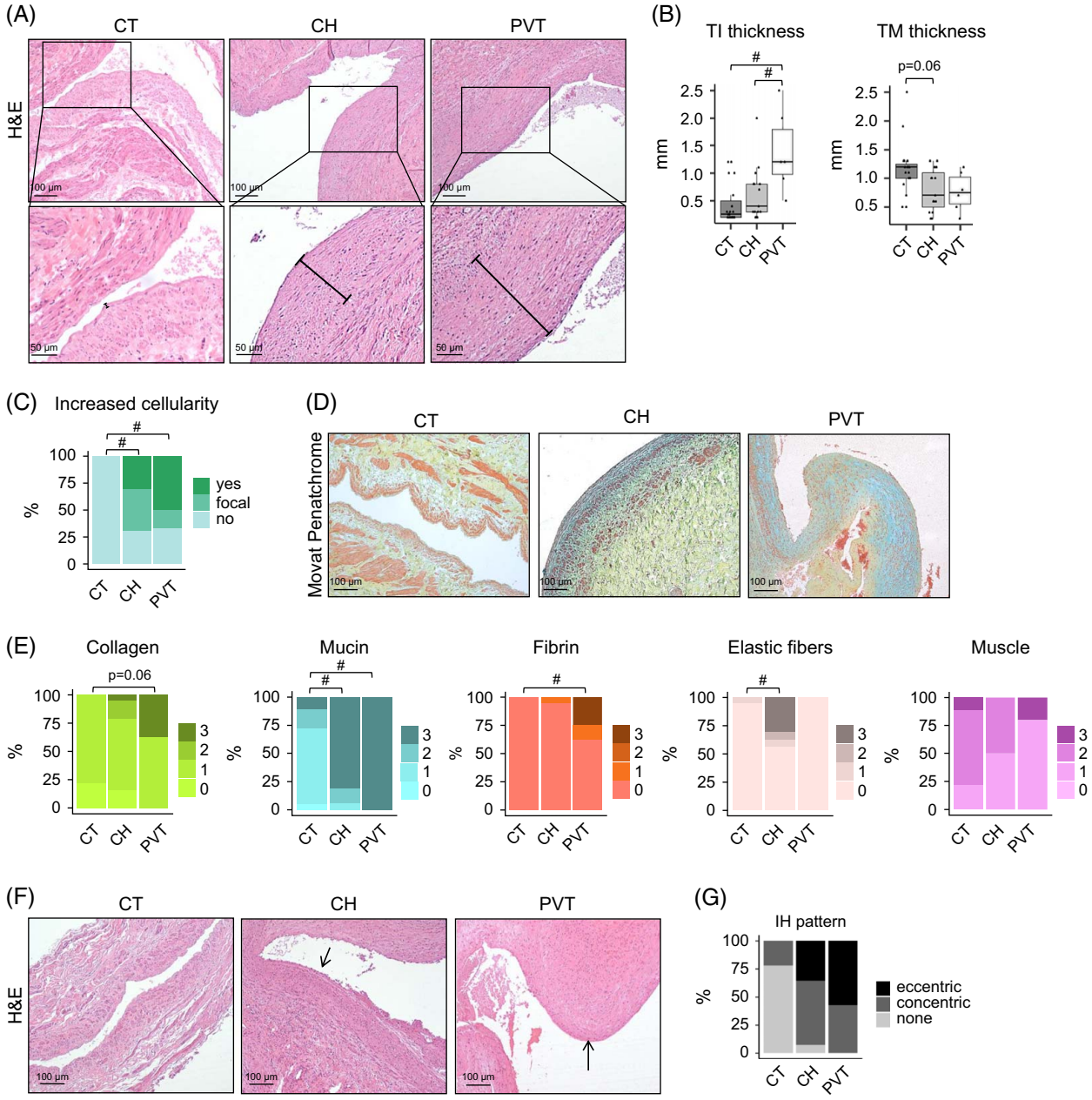

FIGURE 1 Histological analysis of extrahepatic PV (cohort A). Eccentric, heterogeneous, and proliferative neointima in the extrahepatic portal vein. (A) Representative images of the extrahepatic PV wall of H&E-stained sections from human liver donors (n = 19), patients with cirrhosis (n = 15), and patients with cirrhosis and PVT (n = 6). The top row shows 10× magnification images with highlighted regions of interest, while the bottom row displays corresponding 20× magnification views. The black measurement bars in the bottom row denote the thickness of the tunica intima, used for morphological analysis, and the measurements shown in B. Images were acquired using an Olympus IX71 microscope. (B) Measurement of the tunica intima and tunica media in millimeters. Results are displayed as boxplots. All measurements correspond to the maximal thickness observed in each sample. (C) Semi-quantification of increased cellularity within the tunica intima, indicating no increased cellularity, or clear increased cellularity in the tunica intima. Results shown as stacked bars. (D) Movat pentachrome staining of extrahepatic PV, highlighting extracellular matrix components: collagen (yellow to red), elastin (black to blue), muscle (red), mucin (bright blue), and fibrin (bright red). (E) Graphs show quantification of the Movat pentachrome-stained elements as stacked bars. (F) Representative images of the described intimal hyperplasia patterns in the CT, CH, and PVT extrahepatic PV samples. The arrows indicate the concentric intimal hyperplasia in the CH and eccentric intimal hyperplasia in PVT. The control group (left panel) shows no evidence of intimal hyperplasia. (G) Classification of tunica intima hyperplasia as eccentric, concentric, or not present, based on previously established criteria. Results shown as stacked bars. Statistical analysis was performed by the Fisher test or *t* test. (#$p < 0.05$). Abbreviations: CH, cirrhosis patients; CT, liver donor; H&E, hematoxylin and eosin; PV, portal vein; PVT, portal vein thrombosis patients; TI, tunica intima; TM, tunica media.

using the Fisher exact test for small sample sizes and the chi-square test for variables with multiple categories. Multivariate analysis was performed by ANOVA and the Tukey test. Results were considered significant when $p < 0.05$.

The methodology for endothelial cell isolation from human portal vein is described in the recently published work by Anton et al.[23] Additional details regarding RNA extraction, sequencing, and bioinformatics analysis, as well as information on the animal models used, are provided in the Supplemental Material, http://links.lww.com/HEP/K282.

RESULTS

The portal vein wall undergoes marked anatomical changes in cirrhosis

We analyzed extrahepatic PV segments from 14 patients with cirrhosis (CH group), 7 patients with cirrhosis and PVT (PVT group), and 19 liver donors (CT group) (cohort A). Clinical characteristics are reported in Supplemental Tables S1 and S2, http://links.lww.com/HEP/K282.

Histological analysis of PV wall revealed intimal hyperplasia in cirrhosis, characterized by a generalized, though uneven, thickening of the extrahepatic PV wall at the level of the tunica intima (tunica intima thickness: *cirrhosis 0.62 ± 0.5 vs. control 0.44 ± 0.3 mm, p = 0.19*), together with a thinner tunica media (tunica media thickness: *cirrhosis 0.79 ± 0.35 vs. control 1,16 ± 0.45 mm, p = 0.06*) (Figures 1A, B) consistent with the findings by Driever et al.[13] This thickening resulted from tunica intima increased cellularity (Figure 1C) and acellular material deposition, identified by the Movat pentachrome staining. This staining revealed an important accumulation of mucin—indicative of acid mucopolysaccharides and glycosaminoglycans (eg, hyaluronic acid, chondroitin sulfate, and proteoglycans), which have been implicated in pathological vascular remodeling[25]—along with alterations in collagen, fibrin, muscle, and elastic fibers content (Figures 1D, E). These alterations suggest a tunica intima–mediated extracellular matrix remodeling.

We next examined the intimal hyperplasia pattern. While data on the PV are lacking, 2 different patterns of intimal hyperplasia have been described in vessels: concentric and homogeneous,[26,27] which have been associated with normal wall remodeling during life, and eccentric and heterogeneous, which have been linked to pathological conditions such as the occlusion of an arteriovenous fistula.[28] In PV samples from liver donors (CT), we observed some degree of thickening of the TI, which was less prominent, more organized, and exhibited a more concentric pattern compared to the intimal hyperplasia pattern observed in patients with cirrhosis (Figures 1F, G). The concentric thickening presented by controls (outward, lumen-preserving), similarly described in other vascular territories[29–33] may reflect the adaptive response to physiological pressure and volume changes in the splanchnic territory.[34] In contrast, in patients with cirrhosis, the intimal hyperplasia pattern displayed an eccentric and heterogeneous distribution (inward, lumen-narrowing), a pattern commonly associated with maladaptive response to vessel wall injury due to oscillatory and disturbed flow.[35–38]

We next compared PV wall alterations in patients with cirrhosis, with and without PVT. Histological changes were significantly more pronounced in patients with PVT, who exhibited a greater tunica intima thickness (*PVT 1.38 ± 0.69 mm vs. cirrhosis 0.62 ± 0.5 mm*, $p = 0.05$) but similar tunica media thickness (*PVT 0,76 ± 0.36 mm vs. cirrhosis 0.79 ± 0.35 mm*, $p = 0.8$) (Figures 1A–E). The eccentric pattern of tunica intima thickening tended to be more frequent, heterogeneous, and pronounced in the PVT group, suggesting an exaggerated healing response that could potentially narrow the lumen and promote thrombosis (Figures 1F, G).

Analysis of hepatic hilum samples (cohort C, clinical data in Supplemental Table S3 and S4, http://links.lww.com/HEP/K282) revealed similar thickening changes, indicating that this pattern extends throughout the portal vein territory rather than being confined to localized injury (Supplemental Figure S1, http://links.lww.com/HEP/K282).

Cirrhosis is associated with immune cell infiltration in the portal vein wall

Histological analysis identified the tunica intima as the most affected layer in cirrhosis. Since the tunica intima is primarily composed of ECs, we investigated their molecular features. We isolated extrahepatic portal vein endothelial cells (PVECs) using methodology described elsewhere (manuscript under revision) from 3 controls (CT_PVECs), 12 patients with cirrhosis (CH_PVECs), and 5 patients with cirrhosis and PVT (PVT_PVECs) (cohort B) and performed bulk RNA-seq. Clinical characteristics of patients are summarized in Table 1.

Gene Set Enrichment Analysis (GSEA) of PVECS from patients with cirrhosis compared with control samples (CH_PVECs vs. CT_PVECs) revealed that dysregulated pathways were predominantly associated with the activation of the immune response, particularly the innate immunity, together with multiple pathways related to chemokine and cytokine signaling (Figure 2A and Supplemental Table S6, http://links.lww.com/HEP/K283). This finding aligns with studies on graft vascular disease (GVD), showing that innate immune cell recruitment to the vein wall contributes to

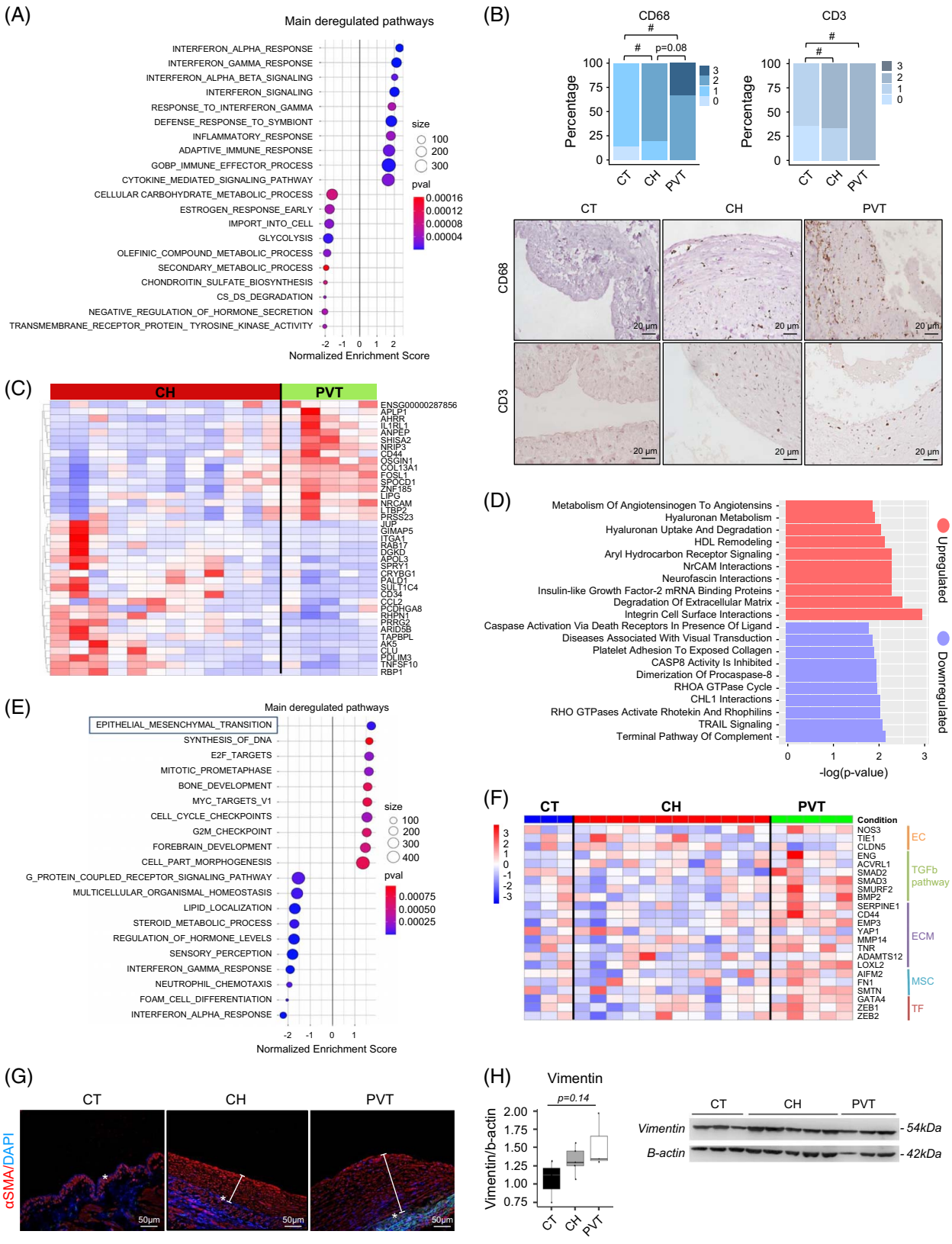

FIGURE 2 Transcriptomic analysis of PVECs (cohort B). (A) Dot plot representing fGSEA results, highlighting the top 10 significantly enriched upregulated and downregulated pathways in CH_PVECs compared with CT_PVECs. (B) Histological CD68 and CD3-stained slides were evaluated for the presence of either macrophages or leukocytes, respectively, in the extrahepatic portal vein wall. Results are shown as stacked bars for each group (CT n = 14; CH n = 15; PVT n = 6). Representative images of CD68 and CD3 staining of extrahepatic PV from CT, CH, and PVT. Images were acquired at 40× magnification using an Olympus IX71 microscope. (C) Heatmap displaying DEGs between CH_PVEC and PVT_PVEC. (D) Bar plot of Enrichr results showing the top 10 upregulated and top 10 downregulated pathways based on DEGs between

CH_PVECs and PVT_PVECs. Pathways are ranked by –log(*p*-value). (E) Dot plot representing fGSEA results, highlighting significantly enriched upregulated and downregulated pathways in PVT_PVECs compared with CH_PVECs. (F) Heatmap of curated EndMT-related genes, categorized into endothelial cell markers (EC), TGFβ pathway components, extracellular matrix (ECM) genes, mesenchymal cell markers (MSC), and transcription factors (TF). (G) Representative images of αSMA staining in the PV wall. The basal lamina, visible in the DAPI channel due to autofluorescence (*), serves as a reference marker for proliferating cells in the tunica intima of CH and PVT patients, marked with white measurement bars. No cells are observed above the basal lamina in controls. Images were acquired at 20× magnification using an Olympus BX41 fluorescence microscope. (H) Western blot analysis of Vimentin protein levels from PVECs of CT, CH, and PVT normalized to β-actin. Results are shown as boxplots for each group (CT n = 3; CH n = 5, PVT n = 3). Statistical analysis was performed by *t* test (#$p < 0.05$). Abbreviations: CH, cirrhosis patients; CT, liver donor; CT_PVEC, portal vein endothelial cells from control patients (polycystic liver disease); CH_PVEC, portal vein endothelial cells from cirrhotic patients without PVT; DEGs, differentially expressed genes; EndMT, endothelial-to-mesenchymal transition; GSEA, gene set enrichment analysis; PV, portal vein; PVECs, portal vein endothelial cells; PVT, portal vein thrombosis patients; PVT_PVEC, portal vein endothelial cells from cirrhotic patients with PVT; αSMA, alpha-smooth muscle actin.

ECM production, cell proliferation, and intimal hyperplasia.[38,39]

Remarkably, no significant alterations in hemostatic pathways were found (Supplemental Table S7, http://links.lww.com/HEP/K284), reinforcing the idea that the splanchnic vascular bed is not a hypercoagulable environment.[20,40]

Based on immune-related alterations in our transcriptomic data and prior reports of immune cell infiltration in vascular diseases,[41–43] we performed immunohistochemical staining for CD68 and CD3 to assess macrophage and T cell infiltration, respectively, in the PV wall. Both cell types play key roles in inflammatory immune response, potentially mediated by IFN-γ, one of the most deregulated pathways in our analysis, and that has been linked to intimal hyperplasia during GVD. Immunostaining confirmed significant infiltration of $CD68^+$ and $CD3^+$ cells in the PV wall during cirrhosis, with even greater increase observed in patients with cirrhosis and PVT (Figure 2B). These findings support the hypothesis of a proinflammatory environment in the PV during cirrhosis, likely contributing to endothelial dysfunction and vascular remodeling.

EC from the tunica intima transdifferentiate into mesenchymal cells in PVT

Given the significant histological changes observed in patients with PVT, we next compared EC from patients with cirrhosis with and without PVT (CH_PVECs vs. PVT_PVECs).

Differential gene expression analysis (Figure 2C and Supplemental Table S8, http://links.lww.com/HEP/K285) followed by pathway enrichment analysis of the differentially expressed genes (Figure 2D and Supplemental Table S9, http://links.lww.com/HEP/K286) revealed multiple molecular alterations between PVT_PVECs and CH_PVECs, primarily related to extracellular matrix remodeling, collagen metabolism, and integrin interactions, alongside deregulated vascular integrity pathways and cytoskeletal alterations, suggesting endothelial barrier restructuring and altered permeability. GSEA comparing PVT_PVECs versus CH_PVECs (Figure 2E and Supplemental Table S10, http://links.lww.com/HEP/K287) identified endothelial-to-mesenchymal transition (EndMT) as the most significantly upregulated pathway in PVT_PVECs, a process previously unexplored in the PV but extensively studied in cardiovascular diseases.[44–46]

Interestingly, no immune or hemostatic alterations were observed among the top dysregulated pathways in the comparison of these 2 conditions (Supplemental Table S11, http://links.lww.com/HEP/K288). Results were consistent when patients with HCC were excluded from the analysis (data not shown).

EndMT is a dynamic process characterized by shifts in gene expression, where cells lose endothelial markers and acquire mesenchymal and ECM-related gene expression. Since EndMT has not been previously characterized in the PV system, and lacks a standardized readout, we conducted an in silico analysis of our sequencing data using literature-established EndMT indicators[47–50] and previously reported EndMT-associated genes. As observed in Figure 2F, PVT_PVECs express higher levels of EndMT-associated genes than CH_PVEC and CT_PVEC. The gene panel shows a homogeneity within the samples in the PVT group in the expression of these EndMT markers, while the cirrhosis and control groups exhibited greater heterogeneity, thus confirming a more pronounced EndMT in PVT.

Histological findings (cohort A) also demonstrated the presence of myofibroblast-like cells (αSMA positive) in PVT samples that were absent in CT (Figure 2G), which, together with a higher expression of vimentin protein in PVT_PVECs (Figure 2H), confirmed the EndMT. EndMT, even if less prominent, also occurred in patients with cirrhosis without PVT, consistent with a progressive nature of the changes, starting in cirrhosis and then amplified in PVT.

Since our PVECs were isolated based on endothelial markers (CD31 and CD144; see Supplemental Material for details, http://links.lww.com/HEP/K282), our findings strongly suggest that the origin of the hypersecretory and hyperproliferative cells producing ECM and driving

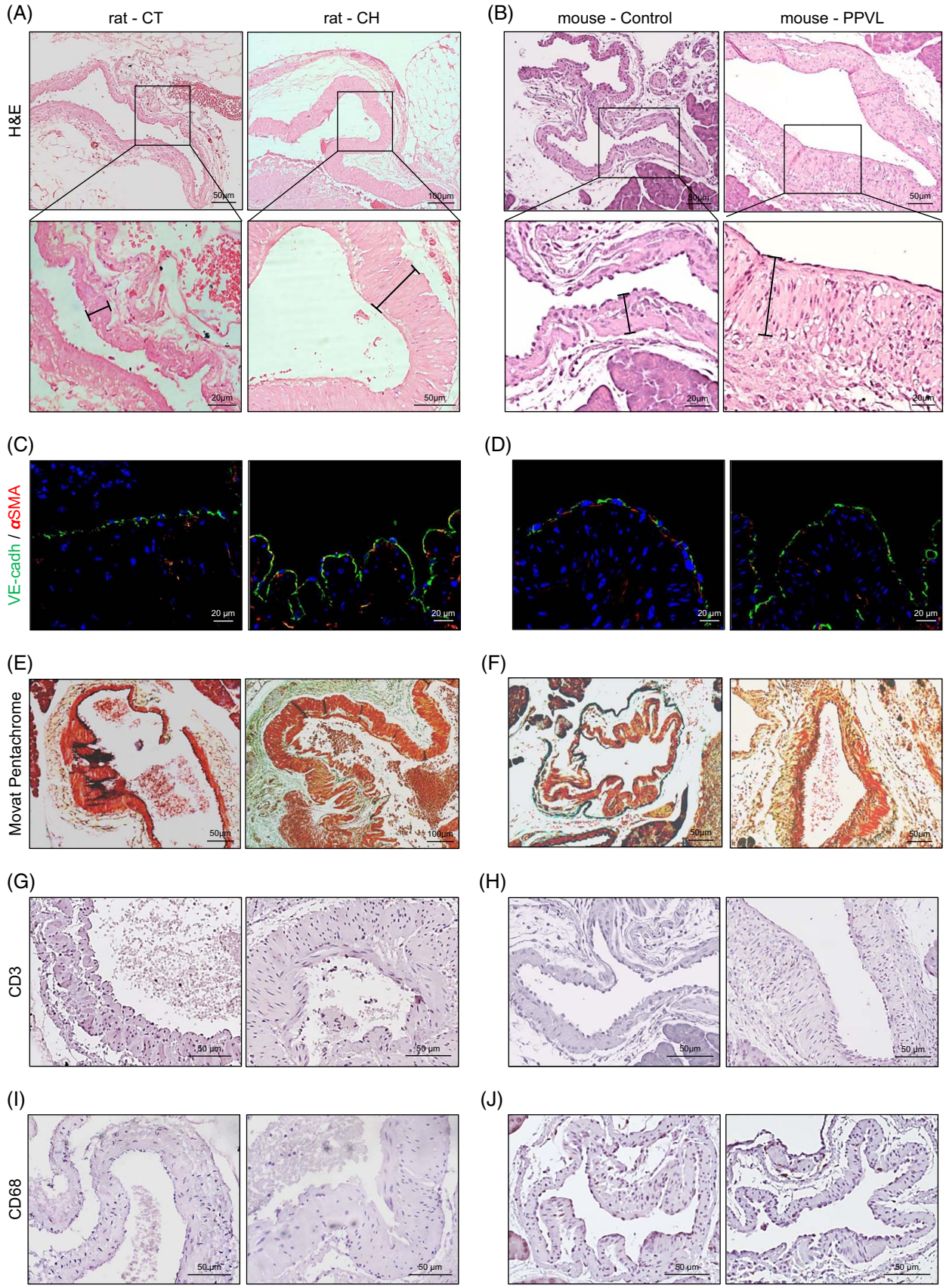

FIGURE 3 Rodent models of PHT do not exhibit either PVT or EndMT. (A) Representative H&E-stained images of the PV from the rat model of CCl_4-induced cirrhosis (CT, n = 5; CH, n = 5) and (B) mouse model of PPVL-induced PHT (CT, n = 3; PPVL, n = 4). The top row presents 20× magnification images with highlighted regions of interest, while the bottom row shows corresponding 40× magnification views. In the bottom row,

black measurement bars indicate the thickness of the entire vein layers. (C) Representative immunofluorescence images of VE-cadherin and αSMA staining in the portal vein of the rat model of CCl_4-induced cirrhosis and (D) mouse model of PPVL-induced PHT. Images were acquired using an Olympus BX41 fluorescence microscope at 40× magnification. (E) Representative images of Movat pentachrome staining of PV from rats with cirrhosis (F) and a mouse with PPVL. (G) Representative images of CD3 staining of PV from rats with cirrhosis (H) and a mouse with PPVL. (I) Representative images of CD3 staining of PV from rats with cirrhosis (J) and a mouse with PPVL. Images were acquired using an Olympus BX41 fluorescence microscope at 20× magnification. Abbreviations: CH, cirrhosis patients; CT, liver donor; EndMT, endothelial-to-mesenchymal transition; H&E, hematoxylin and eosin; PHT, portal hypertension; PPVL, partial portal vein ligation; PV, portal vein; PVT, portal vein thrombosis; αSMA, alpha-smooth muscle actin.

the maladaptive response is, at least in part, ECs from the tunica intima transitioning into myofibroblasts. Although we cannot conclude which role might be playing, the migration of myofibroblasts into the tunica intima compartment, we demonstrate a clear role from the ECs of the tunica intima in the intimal hyperplasia.

Preclinical models of PHT do not develop EndMT or PVT

Due to PV's remote location and invasiveness of sampling, obtaining samples from the early stages of the disease is ethically infeasible and available only at the time of LT. Therefore, we employed rodent models of PHT to study EndMT. Previous studies from the 90's[51] demonstrated pressure-induced thickening of the vessel wall and prompted us to investigate whether PHT induces intimal hyperplasia and EndMT.

In a CCl_4-induced cirrhosis model in Sprague–Dawley rats, the PV wall exhibited concentric thickening and a generalized hypertrophy across all vein wall layers. This thickening primarily resulted from an abnormal accumulation of fibroblasts within the tunica media, forming a prominent muscular layer that, along with vein wall dilation, gave the vein an arterial-like appearance (Figure 3A), suggesting a process of arterialization. Unlike human samples, no EC proliferation was observed in this model, and the tunica intima thickening was notably less pronounced, always displaying a regular and concentric pattern.

We then used a second injury model of PHT, partial portal vein ligation (PPVL) in mice. Similar to rats, PPVL mice exhibited concentric thickening throughout the PV (Figure 3B), but again, it was mainly a muscular hypertrophy with no significant tunica intima thickening or evidence of EC proliferation.

α-SMA staining confirmed the absence of activated mesenchymal cells in the tunica intima of either model, further confirming the absence of EndMT in the PV of rodents with PHT (Figures 3C, D). Moreover, Movat pentachrome staining revealed no evidence of extracellular matrix accumulation in the tunica intima with no presence of mucin in either model (Figures 3E, F and Supplemental Figure S2, http://links.lww.com/HEP/K282), in contrast to the findings in humans.

Finally, neither model displayed infiltrating $CD68^+$ macrophages or $CD3^+$ T cells in the PV wall (Figures 3G–J).

Taken together, these findings suggest that the endothelial response to PHT in the PV of rodents differs from that in humans, which is particularly interesting given that rodents do not spontaneously develop PVT. Our data indicate that PVT development is not solely pressure-driven but shaped by the unique human splanchnic microenvironment.

Drug repositioning analysis reveals statins as potential disease modifiers in PVT

We conducted a drug repurposing analysis by employing the Drug–Gene Interaction Database (DGIdb), which links FDA-approved drugs with associated genes, to identify potential therapies able to target PVT's most deregulated pathways.

Results, shown in Figure 4A, identified atorvastatin as a promising therapeutic agent for PVT; in fact, up to 3 different statins ranked among the top 25 drug candidates (Supplemental Table S12, http://links.lww.com/HEP/K289).

Due to the limitations in manipulating the EndMT pathway in animal models described above, we examined the effect of statins in vitro by treating CH_PVECs (n = 7) and PVT_PVECs (n = 6) with atorvastatin, simvastatin, or vehicle for 24 hours. As shown in Figure 4B, both statins significantly upregulated endothelial markers in PVECs [*PECAM1* (CD31), *TIE1* and *NOS3* (eNOS)] while downregulating the expression of mesenchymal markers (*CDH2*, *AIFM2, SNAI1*). Statin treatment also tended to restore levels of Rho GTPase (*RHOA*) and decrease the expression levels of L-selectin (*PAI1*) while increasing the VEGF expression, suggesting a potential role in ameliorating EndMT.

We next examined our previously published cohort of 369 patients with cirrhosis without baseline PVT who were prospectively followed to detect PVT onset.[20] Over an average follow-up period of 48 ± 27 months, 30 patients (8.1%) developed PVT (Supplemental Tables S13 and S14, http://links.lww.com/HEP/K282). In the overall cohort, only 17 patients received chronic statin therapy, and 1 developed PVT, while 16 did not

(A)

pathway	p-val
Atorvastatin	0.0054
Isoproterenol	0.0092
Fenofibrate	0.0150
Pravastatin	0.0163
Triamterene	0.0184
Azathioprine	0.0223
Nevirapine	0.0230
5-fluorouracil	0.0259
Methimazole	0.0280
Bortezomib	0.0301

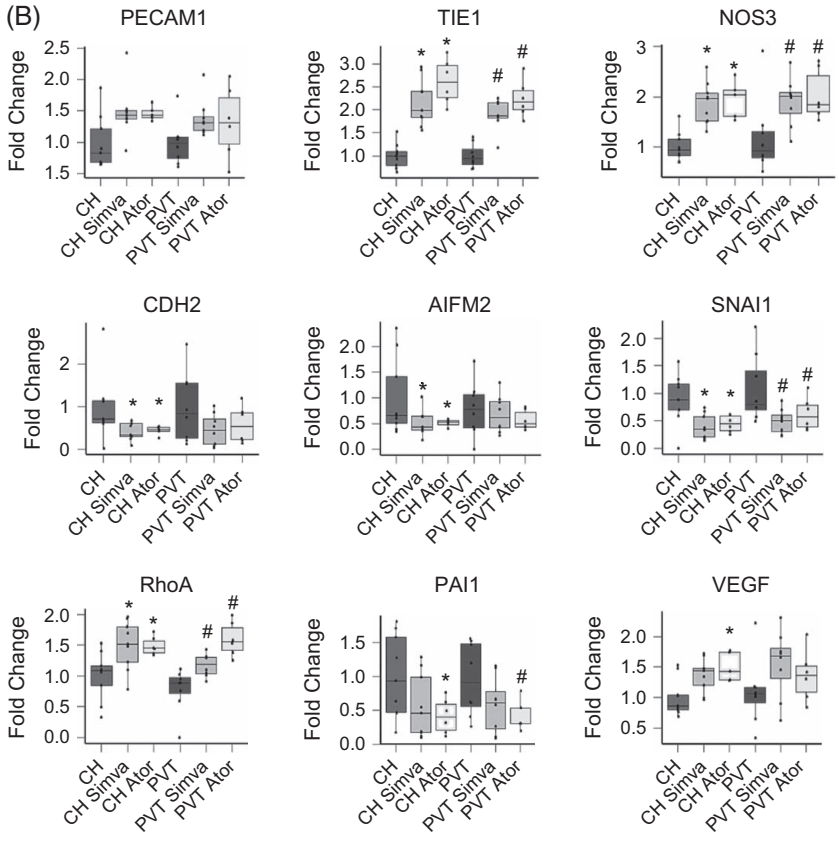

FIGURE 4 Statins ameliorate EndMT markers in PVECs. (A) Drug repurposing analysis. Gene set enrichment analysis comparing CH_PVECs and PVT_PVECs identified potential therapeutic agents using the DGIdb. (B) qRT-PCR analysis of key EndMT hallmark genes [*PECAM1* (CD31), *TIE1*, *NOS3* (eNOS), *CDH2*, *AIFM2*, *SNAI1*, *VEGF*, *PAI1*, and *RHOA]* in PVECs treated in vitro with either vehicle or 5 μM atorvastatin or simvastatin (CH n = 7; PVT n = 6). Gene expression was normalized to *GAPDH*, and results are displayed as boxplots. Statistical analysis was performed using a *t* test (*$p < 0.05$ CH vs. CH-statin and #$p < 0.05$ PVT vs. PVT-statin). Abbreviations: CH, cirrhosis patients; CH_PVECs, portal vein endothelial cells from cirrhotic patients without PVT; DGIdb, Drug–Gene Interaction Database; EndMT, endothelial-to-mesenchymal transition; PVT, portal vein thrombosis patients; PVT_PVECs, portal vein endothelial cells from cirrhotic patients with PVT; PVECs, portal vein endothelial cells; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

(Supplemental Table S13, http://links.lww.com/HEP/K282). This data is in agreement with a recent large observational study reporting a reduced incidence of PVT development during follow-up in statin users.[52] Even if no definitive conclusion can be drawn from these results, they encourage the further study of the role of statins in prospective studies.

DISCUSSION

The contribution of the portal vein endothelium to PVT has been largely overlooked, despite its potential key role in thrombosis development. Endothelial dysfunction may hold the key to identifying novel therapeutic targets to enhance the efficacy of current, predominantly anticoagulant, treatments. Our study provides the first transcriptomic profile of PVECs, revealing EndMT as a central and potentially targetable mechanism in PVT pathogenesis.

Historically, extrahepatic PV remodeling has been associated with PHT, with early autopsy studies linking vessel wall thickening to splenomegaly.[53,54] More recently, Driever et al.[13] proposed that PVT may be driven by intimal fibrosis, which, in turn, seems to be again influenced by PHT.[55] This intimal hyperplasia might potentially explain the PVT resistance to anticoagulation. Our findings build upon this knowledge, demonstrating that, while some degree of concentric intimal hyperplasia occurs as a physiological response to maintain mechanical hemostasis in response to normal flow fluctuations and likely influenced by aging, it becomes pathological in cirrhosis. In this context, intimal hyperplasia progressively becomes exaggerated, eccentric, and extends beyond the thrombosis site. We hypothesize that the eccentric thickening of the tunica intima observed in the cirrhotic PV may result from a maladaptive response to chronic liver injury[38] and its consequences, such as PHT and chronic inflammation. This maladaptive remodeling narrows the PV lumen, likely contributing to blood stasis and thrombus formation.

At the cellular level, we identified intimal EC as key mediators of PVT, marked by increased proliferation and ECM deposition. Isolated PVECs from patients with PVT exhibited a transition toward a mesenchymal phenotype

with secretory activity, consistent with EndMT, suggesting an endothelial-driven pathological remodeling of the PV. No alteration of hemostatic pathways was detected, supporting the notion that PVT is not merely a coagulation disorder[17,20] but rather a maladaptive vascular response to injury. The described alterations were detected both in the extra and intrahepatic portal vein branches, suggesting a global alteration of the splanchnic territory and not limited to the site of the thrombus. Although the precise initiating trigger remains unclear, the presence of inflammatory infiltrates ($CD68^+$ and $CD3^+$ cells) in the PV wall of patients with cirrhosis, along with significant dysregulation of multiple inflammatory pathways in PVECs, suggests that chronic inflammation may drive early endothelial dysfunction and points to the interplay between immune-mediated inflammation and vascular remodeling in the PV during cirrhosis. This aligns with studies in graft vascular disease, where both innate and adaptive immune responses—particularly mediated by IFN-γ—contribute to vascular remodeling.[38] One possible explanation is that cirrhosis triggers an inflammation-mediated endothelial damage response through the activation of EndMT.[56,57] Initially, this would serve as an adaptive wound-healing mechanism, leading to concentric thickening of the tunica intima due to the mesenchymal transformation of endothelial cells.

In this context, EndMT may initially function as a reparative response, contributing to concentric intimal thickening. However, under sustained injury and inflammatory stress, this process becomes pathologic—culminating in aberrant ECM deposition, eccentric thickening, and eventual thrombosis. While causality cannot yet be confirmed, the consistent association between EndMT, intimal hyperplasia, and PVT strongly supports a contributory role for endothelial reprogramming in cirrhosis-associated vascular pathology.

Despite extensive efforts, rodent models of PHT have failed to recapitulate the EndMT and intimal hyperplasia observed in humans, highlighting key interspecies differences in vascular remodeling.[58–60] Instead, these models primarily develop smooth muscle hypertrophy,[61,62] suggesting an arterial-like adaptation to increased pressure rather than the endothelial-driven changes seen in humans. The absence of significant immune infiltration further highlights key interspecies differences in vascular remodeling and may explain why spontaneous PVT has not been observed in rodents.

EndMT is potentially reversible, and emerging evidence suggests that pharmacological interventions can modulate this process.[63–65] Our in silico analysis identified statins as promising candidates targeting dysregulated pathways in PVECs. In vitro, atorvastatin and simvastatin reversed EndMT markers, promoting an endothelial phenotype while reducing mesenchymal-associated gene expression. Given their well-established safety profile in cirrhosis and emerging benefits in improving hepatic microcirculation and reducing PHT,[66–69] along with their pleotropic effects, such as anti-thrombotic, anti-inflammatory, and vasodilatory properties that may help restore endothelial dysfunction, statins represent a therapeutic strategy worth exploring for PVT. However, a clinical trial will be necessary to confirm whether the observations described herein ultimately translate into a reduced risk of PVT.

Statins can reduce portal pressure in patients with cirrhosis by improving endothelial function and reducing proinflammatory cytokines.[70] Most recently, statins use has also been associated with a reduced incidence of HCC in this population,[71] potentially by mitigating the progression of liver fibrosis by mitigating the risk of worsening liver fibrosis. However, the specific mechanism of action by which statins may ameliorate EndMT and their broader implication for PVT treatment deserve further investigation.

While our study provides novel insights, several limitations are acknowledged. First, our cell purification method, based on CD31 and VE-cadherin markers, may bias the analysis toward cells in early EndMT stages due to marker loss in more advanced transitions. However, despite this potential limitation, we detected significant differences between patients with and without PVT, supporting the robustness of our findings. Second, all samples were obtained from liver transplant recipients, which may limit the generalizability of the results. Given the invasiveness of PVEC sampling, however, alternative patient cohorts are challenging to access. Finally, while our data suggest a strong association between inflammation, EndMT, intimal hyperplasia, and PVT, the causal relationship remains to be fully elucidated.

In conclusion, our data position PVT as an endothelial-driven vascular complication of cirrhosis, revealing the role of changes in the portal vein wall in the development of PVT. EndMT emerges as a key mechanistic link between chronic injury and maladaptive intimal remodeling. We propose for the first time that EndMT in endothelial cells of the portal vein during liver disease contributes to pathological intimal hyperplasia, ultimately leading to luminal narrowing and thrombosis. Our findings underscore the potential of statins as EndMT modulators in the portal vein, with the potential of preserving vascular integrity and preventing thrombosis. The beneficial effects of statins in PVT prevention should be confirmed in long-term clinical trials.

Further clinical and translational investigation is warranted to validate this pathway and assess its potential as a treatment target in cirrhotic vasculopathy.

DATA AVAILABILITY STATEMENT

RNA-seq data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus [GEO; accession numbers GSE261883 (newly generated data) and GSE296600 (from our previously published paper https://doi.org/10.1016/j.jhepr.2025.101543)].

AUTHOR CONTRIBUTIONS

Aina Anton contributed to study design, conceived ideas, conducted experiments, performed data analysis and bioinformatics, and wrote the manuscript. Sarah Shalaby conducted experiments, performed clinical data analysis, contributed to the result discussion, and manuscript writing. María Luisa Botero performed the histopathological analysis. Aina Anton, Gonzalo Crespo, Annabel Blasi, Yiliam Fundora, Pol Olivas, and Gonzalo Crespo contributed to sample collection. Carla Montironi contributed to the histopathological analysis. Sergio Barace contributed to RNA-seq bioinformatics analysis and shared pipelines. Rosa Montañés, Héctor García-Calderó, Maria Velasco-de Andrés, and Hasanka Madubashetha performed experiments. Lara Orts, Cynthia Bazán, and Rommel Zambrano-Huailla provided clinical data. Peio Aristu Zabalza contributed to animal studies. Josepmaria Argemí supervised RNA-seq bioinformatics analysis and results discussion. Scott L. Friedman contributed to the results discussion and critical manuscript revision. Juan Carles García-Pagán provided economic support, results discussion, study guidance, and critical manuscript revision. Gonzalo Crespo contributed to study design, conceived ideas, conducted experiments, performed RNA-seq bioinformatics, data analysis, and manuscript writing. Virginia Hernández-Gea designed the research, conceived ideas, wrote the manuscript, obtained funding, and directed the study. All authors edited and reviewed the final manuscript.

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CONFLICTS OF INTEREST

Juan Carles García-Pagán advises GSK. He consults for and is on the speakers' bureau for Cook Medical and Gore Medical. He consults for and received grants from AstraZeneca. Virginia Hernández-Gea received grants from Cook Medical and Gore Medical. The remaining authors have no conflicts to report.

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